

RECEIVED

APR 27 1990

TECH CENTER

B1  
-- Figure 2A shows a schematic diagram of the DT mutants CRM9, CRM197 and MSPΔ5. The A- and B-subfragments and their relative size and position are shown. The filled circle represents a point mutation as described in the text.

Figure 2B shows the inhibition of toxicity by anti-DT goat serum incubated with CRM197 (-O-), MSPΔ5 (-X-) or the B-subfragment (-Δ-) of DT.

B  
Figure 2C shows the inhibition of toxicity by anti-DT human serum pooled from all samples with positive ELISA for anti-DT antibodies. In each experiment, the goat and human sera were separately incubated with increasing molar concentrations of CRM197 (-O-), MSPΔ5 (-X-) or the B-subfragment (-Δ-) of DT for 30 minutes at room temperature. To this reaction, UCHT1-CRM9 was added to a final concentration of  $1 \times 10^{-10}$  M. This mixture was then diluted 10-fold onto Jurkat cells in a protein synthesis inhibition assay as described in the Materials and Methods. Immunotoxin incubated with medium only inhibited protein synthesis to 4% of controls. The results are representative of two independent assays. The data show the epitopes involved in human serum's inhibition of toxicity lie in the last 150 amino acids of DT.--

Page 5, line 18, through page 5, line 27, delete the description of Figure 3 and insert therefor the following:

-- Figure 3A shows the effect of increasing concentrations of sFv-DT390 (-Δ-) or UCHT1-CRM9 (-O-) in protein synthesis inhibition assays as described in the Materials and Methods. The results are an average of four separate experiments. sFv-DT390 maintains specificity for the CD3 complex but is 16-fold less toxic than UCHT1-CRM9 to Jurkat cells.

Figure 3B shows the effect of increasing concentrations of UCHT1 antibody mixed with a  $1 \times 10^{-10}$  M UCHT1-CRM9 (-O-) or  $3.3 \times 10^{-10}$  M sFv-DT390 (-Δ-) and then added to cells for a protein synthesis inhibition assay. sFv-DT390 maintains specificity for the CD3 complex but is 16-fold less toxic than UCHT1-CRM9 to Jurkat cells.--

✓ Page 6, line 16, after "Fig. 6" insert --shows--.

Page 6, line 33 through page 7, line 7, delete the description of Figure 8 and insert therefor the following:

--Figure 8A shows the TNF- $\alpha$  secretion induced by UCHT1 and scUCHT1. scUCHT1 had little effect on TNF- $\alpha$  secretion. scUCHT1 from both COS-7 (-Δ-) and SP2/0 (-O-) cells and UCHT1 ( $\square$ ) were added to cultures of human blood mononuclear cells. Culture supernatant was harvested and used for ELISA determination of TNF- $\alpha$ .

3  
B cont.  
Figure 8B shows the IFN- $\gamma$  production induced by UCHT1 and scUCHT1. scUCHT1 inhibited the basal production of IFN- $\gamma$ . scUCHT1 from both COS-7 ( $\Delta$ ) and SP2/0 (O) cells and UCHT1 ( $\square$ ) were added to cultures of human blood mononuclear cells. Culture supernatant was harvested and used for ELISA determination of IFN- $\gamma$ .

✓ Page 19, line 1, delete "caboxy" and insert therefor --carboxy--.

✓ Page 28, line 31, delete "Conjuqates" and insert therefor --Conjugates--.

✓ Page 29, line 4, delete "desireable" and insert therefor --desirable--.

Page 38, line 36, after "diphtheria toxin" insert --avoids inhibition by pre-existing  
B4 antibodies in human blood.--

✓ Page 39, line 21, delete "singe" and insert therefor --single--.

✓ Page 41, line 29, delete "(Gly<sub>3</sub>Ser)<sub>4</sub>" and insert therefor --(Gly<sub>4</sub>Ser)<sub>3</sub>--.

✓ Page 46, line 19, delete "resultd" and insert therefor --resulted--.

✓ Page 49, line 14, delete "(Gly<sub>3</sub>Ser)<sub>4</sub>" and insert therefor --(Gly<sub>4</sub>Ser)<sub>3</sub>--.

✓ Page 53, line 23, delete "reatments" and insert therefor --treatment--.

✓ Page 56, line 29, delete "tanscription-translation" and insert therefor --transcription/  
translation--.

B<sub>5</sub> ✓ Page 57, delete Table 6 and insert therefor new Table 6 as shown on the replacement page

57.

✓ Page 64, line 3, delete "sepecificity" and insert therefor --specificity--

✓ Page 64, line 28, delete "sequenc" and insert therefor --sequence--

✓ Page 64, line 33, delete "dwonstream" and insert therefor --downstream--.

✓ Page 64, line 35, delete "full-lenth" and insert therefor --full-length--.

✓ Page 67, line 6, delete "herin" and insert therefor --herein--.

B<sub>6</sub> On pages 74-76, please delete the Sequence Listing and insert therefor the following new  
Sequence Listing attached.

IN THE CLAIMS

Please add new claims 21-42, as follows:

B<sub>7</sub> --21. A fusion immunotoxin, comprising a single chain variable region of an anti-CD3  
antibody linked to a toxin moiety. D